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THE ISOLATION OF DIMETHYL SULFOXIDE SOLUBLE COMPONENTS FROM HUMAN EPIDERMAL PREPARATIONS: A POSSIBLE MECHANISM OF ACTION OF DIMETHYL SULFOXIDE IN EFFECTING PERCUTANEOUS MIGRATION PHENOMENA*

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ABSTRACT

The present findings indicate that dimethyl sulfoxide is a useful solvent for the extraction of biological material from human epidermal preparations at physiological temperatures. Lipoproteins and nucleoproteins were the main products found and it is suggested that chemical extraction may contribute to dimethyl sulfoxide action in enchancing the percutaneous migration rates of a wide variety of compounds.

The ability of dimethyl sulfoxide (DMSO) to enhance the percutaneous migration of a wide range of compounds *in vivo* and *in vitro* is well documented (1–4). Nevertheless no satisfactory explanation exists to describe the absolute mechanism by which DMSO achieves this effect even though many investigations have been carried out and a number of theories put forward (5–7). As a result of previous investigations performed in our laboratories we have been concerned that apart from its potential physical effects on the skin structure as exemplified by swelling phenomena (5), DMSO may also function as a chemical extractant.

The present report outlines a series of studies aimed at determining the classes of biologically significant materials which we removed from samples of isolated human epidermis following incubation with DMSO under various conditions.

MATERIALS AND METHODS

Preparation of skin samples. Human skin was obtained from the abdominal region of females at autopsy. Specimens were in the age range 55–75 years and skin samples were stored at −20° C until required. Epidermal membranes were isolated by the procedure of Kligman and Christopher (8) although treatment with trypsin solutions was omitted to avoid possible damage to the stratum corneum. Conventional histological examination (9) indicated that samples prepared carefully in this way contained relatively few adherent layers of Malpighian cells.

Dimethyl sulfoxide extraction procedure. Mixed samples of ambiently hydrated isolated epidermal membranes were immersed in freshly distilled 100% DMSO in the proportions 1 gram of tissue per 20 ml of solvent. A sample of tissue was dried to a constant weight at 100° C over $CaCl_2$ *in vacuo* and the degree of hydration determined to permit expression of the results obtained on a dry weight basis. The suspension of isolated epidermis in DMSO was maintained at 37° C for 4 hours with intermittent agitation. The solution was cooled and filtered through a glass-wool plug and further clarified by passage through a fine pore Gooch filter. The clear yellow filtrate was stored at 0° C until required.

The residue of isolated epidermis was resuspended in a further volume of 100% DMSO, again in the proportions 1 gram of tissue per 20 ml of solvent and maintained for 4 hours at 60° C. The filtrate was recovered as described above.

The filtrates from the 37° C and 60° C treatments were reduced to dryness by distillation *in vacuo* at 55° C and resultant residues weighed. Both products were resolvable into two major fractions, one being soluble in chloroform-methanol (2:1 v/v) and the other in distilled water. The materials solubilized in these fractions were recovered by rotary evaporation *in vacuo* at 37° C weighed and subjected to analysis. A small amount of insoluble, denatured material was discarded.

Chemical analysis of extracts. Total nitrogen values were determined by the micro-Kjeldahl method and protein by the Folin-Lowry procedure (10). Carbohydrate was assayed by the anthrone method (11). The lipid content of the chloroform-methanol soluble fraction was determined by direct weighing of dried hexane extracts after hydrolysis in 2N methanolic-HCl at 100° for 4 hours.

Physical analysis of extracts. Ultra-violet absorption spectra were determined in 1 cm silica

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cells in a D-B Spectrophotometer (Beckman Instruments) employing automatic scanning and recording apparatus. Thin-layer plates of silica gel, 25 μ thickness were prepared from Keiselgel-G applied as a suspension in distilled water (1:2 w/v). Spots were visualized with ninhydrin in acetone (0.5% w/v), rhodamine-6G aqueous solution (0.001% w/v) and concentrated sulphuric acid. Detection of ultra-violet sensitive areas was achieved by viewing under a direct beam from an ultra-violet lamp (Hanovia Products Ltd.).

RESULTS

The results obtained after extraction of duplicate samples of isolated epidermal membranes with 100% DMSO at 37° C and 60° C are shown in Table I. Approximately 7.5% of the total dry weight was removed following treatment at 37° C with a further 7.5% being removed at the subsequent 60° C extraction although markedly less nitrogenous material was present in the latter.

Resolution of the 37° C and 60° C extracts into chloroform-methanol soluble components and water soluble components indicated that the former solvent accounted for the greater proportion by weight of both extracts. The results obtained following analysis of these fractions are shown in Table II. There was an overall similarity between material extracted at 37° C and 60° C particularly with respect to the ratios of protein: carbohydrate: lipid. Both water soluble fractions contained a high proportion of protein and the ultraviolet absorption maxima at 260 nm suggested the presence of nucleoproteins.

A small percentage of carbohydrate was present in both 37° C and 60° C water soluble extracts.

Analysis of the chloroform-methanol soluble extracts following 37° C and 60° C treatments revealed that a significant proportion of protein was present in each instance. The solubility characteristics suggested that in both fractions material was present of a lipoprotein nature. Carbohydrate was not detected in the lipid fractions.

The chloroform-methanol soluble components of both 37° C and 60° C extractions were subjected to examination by thin-layer chromatography. No resolution of the constituents occurred when a developing solvent of hexane-benzene (1:1 v/v) was employed and the extracts remained immobile at the origin. A successful separation was achieved in a butanol-acetic acid-water solvent after development for 1 hour. Visualization under ultraviolet light revealed four major zones of fluorescence. The bulk of the applied fluorescent material remained at the origin indicative of highly polar lipid material. Further zones of fluorescence were observed partitioned between the origin and the solvent front and clearly corresponding to less polar material. Staining of the butanol-acetic acid-water developed thin-layer plates with rhodamine 6-G followed by examination under ultraviolet light revealed the presence of three further zones of ultraviolet sensitivity including a dark purple band.

In hydrolysis studies samples of chloroform-methanol soluble fractions were hydrolyzed in

TABLE I

Total extracts obtained in 37° C and 60° C DMSO treatments

Sample	Wt. of dry tissue (grams)	Percentage of isolated epidermis (on dry wt.)	Total nitrogen (%)
Isolated epidermis	3.30	—	12.3
37° C DMSO extract	0.248	7.5	8.7
60° C DMSO extract	0.252	7.6	6.2

Results represent the mean value of duplicate experiments.

TABLE II

Analysis of 37° C and 60° C DMSO extracts

	Chloroform-methanol fractions		Water-soluble fractions	
	37° C	60° C	37° C	60° C
Wt. of extract (mg)	201	207	37.7	41.0
Total nitrogen (%)	8.2	4.2	9.8	10.8
Protein (%)	28.2	32.6	87.0	85.6
Carbohydrate (%)	0	0	2.5	1.6
Lipid (%)	47.5	53.0	—	—
U.V. absorption maxima (nm)	275, 235 300	280	260	260
U.V. 260/280 ratio	1:27	1:22	1:12	1:8

Results represent the mean value of duplicate experiments.

Percentages are based on dry weight of extract.

methanolic-HCl and hexane extraction of the products yielded lipid content values shown in Table II. Hexane extracted material was ninhydrin negative and behaved as non-polar lipid in both thin-layer chromatography solvents described above. These hexane soluble materials however retained their fluorescence characteristics under ultraviolet light and gave complex absorption spectra with maxima at 260–280 nm and 300 nm. Aqueous extracts after acid hydrolysis were strongly ninhydrin positive, anthrone negative and possessed well defined ultraviolet absorption maxima at 280 nm indicating the major presence of a protein component.

DISCUSSION

Although a complete characterization of the materials extracted from human epidermal preparative using DMSO is beyond the scope of the present communication the results obtained indicate that the major components extracted were lipoproteins and nucleoproteins. The bulk of the lipid fraction was of a highly polar nature. Free fatty acids and less polar lipids were also considered to be present in the DMSO soluble extract although their origin whether from sebum, native or degraded components of the isolated epidermal membranes is uncertain.

The use of DMSO as an extraction solvent has been shown previously by Adams (12) who isolated lipopolysaccharide and protein from bacterial cell-wall preparations. The results obtained in the present study demonstrate that the solvent effectively removes polar lipids of epidermal origin. It is also considered that the broad solvent properties of DMSO may permit the removal of polar lipids more representative of the native constituents of isolated epidermal membranes than has hitherto been achieved with other lipid solvents (13).

Since DMSO is now shown to remove substantial amounts of polar lipid at a physiological temperature it is possible that lipid extraction is a mechanism operative in reducing the barrier function of human skin towards a variety of substances following treatment with this agent. Studies carried out by Embery and Dugard (11) using DMSO pretreated epidermal membranes when examining the percutaneous migration of alkyl ^{35}S-sulfates may particularly reflect this situation. On the other hand it is difficult to relate the extremely rapid changes of barrier function as traced by electrical impedance measurements (14) to a mobilization or extraction of polar lipids. Further evidence for a mechanism of action of DMSO on stratum corneum other than extraction of lipids, is the high degree of reversibility of the reducton of water barrier function following a pretreatment *in vivo* (15).

An alternative theory of DMSO action is proposed by Elfbaum and Laden (5) who suggest that DMSO functions as a swelling or unfolding agent on certain soluble proteins and conclude that alterations in primary structure are responsible for changes in the barrier function of stratum corneum under the influence of this solvent. The possibility that DMSO alters the fibrillar content of stratum corneum cells is also supported by ultrastructural evidence (16) although the staining and apparent structural changes are difficult to interpret. It is also considered that reversible configurational changes in protein structure resulting from the substitution of integral water molecules by dimethyl sulfoxide may also occur (7).

To conclude it would appear unlikely that any one mechanism is responsible to explain the action of DMSO in relation to its role in percutaneous migration studies. A number of possible contributary factors have been listed above. To these must be added to likelihood that DMSO functions as a chemical extractant in increasing the migration rates of a variety of given substances following their application to skin samples in the presence of this agent.

SUMMARY

Samples of human epidermis were obtained from female abdominal autopsy skin specimens by treatment at 50° C. Careful use of this technique resulted in the isolation of tissue comprising stratum corneum cells with relatively few viable epidermal cells adherent. This material was extracted at 37° C for 4 hours and subsequently at 60° C for 4 hours using freshly distilled 100% DMSO and resulted in the removal of 15% of the original material on a dry weight basis.

Analysis of the 37° C and 60° C extracts revealed that both could be resolved into chloroform-methanol and water soluble fractions the former solvent accounting for the greater proportion by weight of each extract. Chemical analysis indicated that the chloroform-methanol

fractions contained a high proportion of polar lipid which possessed the characteristics of lipoprotein. The lipid could be resolved into a number of fluorescent and rhodamine sensitive areas on thin-layer chromatograms. The water soluble fractions were composed of nucleoprotein material.

The possibility of DMSO functioning as a chemical extractant in relation to its role in enhancing the percutaneous migration rate of a number of substances is discussed.

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